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THE STABILITY OF BIOLOGICAL PROPERTIES IN THE ANTHRAX STI-1 VACCINE STRAIN

[Following is the translation of an article by N. N. Ginsburg, R. A. Saltkov and V. R. Arkhipova, Tarasevich State Control Institute for Medicinal Biological Preparations, published in the Russian-language periodical Zhurnal Mikrobiologii, Epidemiologii i Immunobiologii (Journal of Microbiology, Epidemiology and Immunobiology), #9, 1964, pages 104-107. It was submitted on 28 Mar 1963. Translation performed by Sp/7 Charles T. Ostertag Jr.]

For more than 20 years the STI-1 vaccine strain, isolated in 1940 (Ginsburg, 1946), has been used in the production of live anthrax vaccine for the immunization of people and farm animals in the USSR. In our country the great amount of experience which has been accumulated in the area of studying live vaccines testifies to the fact that in vaccine strains, during the process of their being maintained in a laboratory accompanied by periodic reseeding, their biological properties change. For practical purposes it is particularly important that along with this there may be a lowering in the immunogenicity of the culture. Such observations in respect to tularemia vaccine strains Ondatra, No 10, 15, 33 and 53 have been described by Faybich and Tamarina (1947), Motornaya (1953), Yemelyanova (1957) and others. There is data concerning the lowering of immunogenicity also in the plague vaccine strains Tjiwidej Otten, EV, No 1 and 17 (Korobkova, 1956, 1961). The stated changes of the strains usually find a reflection also in the nature of their growth on nutrient media -- in the so-called dissociation of the cultures.

As is known, the relative stabilization of the properties of the bacterial cultures is guaranteed when they are converted into an anabiotic state by means of desiccation in a deep vacuum from a frozen state. Natural anabiosis, setting in during the conversion of bacilli into a spore form, still further promotes the prolonged preservation of the biological properties of microorganisms. However, the periodic conversion of spores into the vegetative form during the reseeding of cultures leads to genesis in the population and to the possible accumulation in the appropriate conditions of new variants, in particular nonimmunogenic, if the question concerns the vaccine strain. We see facts of this order in the changes in the biological characteristics of the anthrax vaccine strains Tsenkovskiy and Tamarina strain No. 3 (Arkhipova, 1961).

In the production of live anthrax vaccine, standard cultures are used which are suspensions of spores of a vaccine strain in a 30% aqueous solution of glycerin. With each new preparation of standards for a vaccine strain, they are checked according to the indices characterizing the strain. However, at the Tarasevich State Control Institute during a study of the standards for the STI-1 vaccine strain which has been used in recent years, a significant change was established in the nature of growth of this strain in comparison with its growth in the first years following isolation (Ginsburg, 1946; Saltykov, 1946; Kopylov, et al, 1946). In seedings on Hottinger nutrient agar of the standard culture for 1957, and to a greater degree the standard of 1960, an increased amount of RO-forms of colonies was observed, and in seedings in broth -- a tendency for diffuse growth with turbidity of the medium.

In as much as we had the assignment of preparing a new standard for the STI-1 vaccine strain, we, taking into consideration the appearance in cultures of this strain of forms of growth which are atypical for anthrax bacilli, decided to turn to a study of early generations of the strain, which for a long time had been preserved in the spore form with the minimum number of reinoculations. With this aim we selected a spore culture of the STI-1 strain (3rd generation on an agar medium) which had been desiccated by the lyophile method in 1943. Up until the beginning of the investigation (in the end of 1961) the stated dry spore culture had been preserved for 18 years in a refrigerator. The temperature was not always constant but it was low (lower than 0°). Due to the technical imperfection of vacuum drying for those years, by the time of our investigation the vacuum was not preserved in the ampule with the culture.

During the study of the morphology and nature of growth of subcultures obtained from the 1943 dry culture, it was established that there was greater conformity of their features to those of the original STI-1 strain than there was in the features of the 1957 and 1960 standards. On the basis of these preliminary facts, a 4th generation of the strain was obtained from the stated culture of the 3rd generation by means of incubation on solid nutrient medium. The 4th generation, which was in the form of an aqueous spore suspension, was dried by the method of lyophilization in ampules. Each ampule contained four billion spores (calculation in a Goryayev Chamber). The resulting series of dry spore culture was subjected to a comprehensive study as the proposed new standard for the STI-1 vaccine strain.

The dry 4th generation culture of the STI-1 strain contained 96% live spores (calculation by the method of microcultures). In seedings on broth, the cottony growth typical for anthrax bacilli was observed on the bottom of the vessel without turbidity of the stratum of the medium. In dishes with Hottinger nutrient agar, colonies of the R-RR0 form pre-

dominated; no more than 1% of RO-forms were encountered. In the organs of inoculated white mice and on special serumal media, only the non-encapsulated bacilli, typical for the strain, were always detected. Following the subcutaneous administration of 10 million spores to 10 white mice, the formation of edemas was observed in all the animals and two of the 10 mice died. Following the subcutaneous administration of an aqueous suspension containing from one up to 50 million spores to guinea pigs, local edemas were observed in all the animals (++ -- ++++); three out of 29 died without the symptoms of septicemia. Following the subcutaneous administration of 250 million spores to rabbits, all the animals remained alive and the formation of edemas was not observed in them.

Thus, based on cultural-morphological features and the degree of reactogenicity for laboratory animals, the culture under study fully conformed to the initial STI-1 strain.

The preliminary test for checking the immunogenicity of the strain was set up on guinea pigs. The animals were immunized one time subcutaneously with a spore suspension in doses from one million up to 50 million spores. The vaccinated pigs were infected on the 21st day with a selected variant of the 2nd vaccine of Tsenkovskiy No. 71/12, which is virulent for guinea pigs. The infection dose comprised one million spores, which corresponds to 200 DL for pigs.

The test showed (see table) that 10 million spores of the vaccine culture protected the inoculated pigs from subsequent infection with a culture which was virulent for them.

On the basis of the results of the laboratory investigations, the dry 4th generation culture of the STI-1 strain was proposed for commission approval as the new standard culture of this vaccine strain. In June of 1962, the enlarged commission of the Tarasevich State Control Institute, at a plant of the Orlovskaya Biologicals Factory, carried out an experimental check of the reactogenicity and immunogenicity of the standard culture in tests on rabbits and sheep.

Footnote: The commission was composed of R. A. Saltykov, V. R. Arkhipova, N. N. Ginsburg, S. G. Kolesov, I. N. Presnov, Ye. S. Nikolayev, A. G. Mamayev and A. G. Mamchich.

In the test conducted by the commission, 12 chinchilla rabbits weighing 2--2.5 kg and 17 sheep were used. The rabbits were inoculated

one time subcutaneously with an aqueous suspension of spores of the vaccine culture being tested. This was introduced in the amount of 250 million spores. An insignificant edema condition was observed temporarily at the site of injection on the animals. In 14 days following the vaccination, the rabbits were infected with the highly virulent Ch-7 anthrax culture. The animals received 2000 spores which comprised 10 Dcl for this species of animal. All the vaccinated rabbits survived following infection, along with the death of the control, non-vaccinated rabbits in the course of 48-50 hours.

The sheep were also inoculated one time subcutaneously with a suspension of the STI-1 standard culture in a dose of 12.5 million spores. In the course of a 14-day observation in 3 out of 7 vaccinated sheep there was noted a one day increase of temperature from 40 up to 40.4°. In 14 days the vaccinated sheep were infected intracutaneously with a virulent culture of the Ch-7 strain. The Dcl of this strain was determined preliminarily and was equal to 1,000 spores. The animals received 10,000 spores, which corresponded to 10 Dcl. All the vaccinated sheep remained alive after infection. On the third day in one of the sheep there was a one day increase of temperature up to 41.5°. The control, nonvaccinated sheep, out of which five were infected with 10 Dcl and four with 5 Dcl, died in periods from 48 up to 72 hours after infection.

The commission, having noted the insignificant reactogenicity and high immunogenicity of the dry spore culture of the STI-1 strain being tested for rabbits and sheep, recommended that it be used as the standard for the production of live anthrax vaccine for medical purposes.

Final testing of the reactogenicity of the standard culture of the STI-1 strain for 1962 was conducted on volunteers who were vaccinated by the method of scarification. With this aim, five volunteers were inoculated cutaneously by the usual method of vaccination with live STI vaccine, but with the use of a spore suspension of increased concentration, containing 8 billion spores in 1 ml instead of the 2 billion/ml in the standard dose. There was no general reaction to the inoculation in the volunteers and no complications. The local reaction was expressed more sharply, which is apparently connected with the increased concentration of vaccine.

Conclusions

1. In cultures of the STI-1 strain, which over a period of 20 years following isolation were subjected to periodic reinoculation and storage in 30% glycerin, signs of dissociation were observed. These were expressed in the appearance of the nature of growth on solid and liquid nutrient media which was not typical for the anthrax microbe.

2. A 3rd generation spore culture of the STI-1 strain, which had been stored in a desiccated (lyophilized) form for 18 years, preserved all the biological features characteristic for the strain, both in regards to type of growth on nutrient media as well as in regards to reactogenicity and immunogenicity for animals.
3. Storage of spore cultures of a strain -- standards in a desiccated state, must be recognized as the optimum conditions for the stabilization of the properties of the strain.
4. On the basis of the data from the study of the new standard STI-1 vaccine strain (1962) it must be recommended that this standard culture be used for the production of live anthrax vaccine, and also for scientific-research work, using it to replace all the other cultures (lines) of this strain being used in various laboratories.

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Immunogenicity of a culture of ST1-1 (4th generation) in a test on guinea pigs.			
Dose of vaccine culture (millions of spores)	Number of pigs	Results of infection with a virulent culture	
		Survived	Died
1	3	1	2
10	4	4	0
20	4	4	0
50	5	5	0
0 (Control)	3	0	3